SURFACE TREATMENT

The present invention generally relates to surface treatment and particularly relates to methods for coating surface areas with molecular monolayers.

5 Molecular monolayers on surfaces are useful in many applications, including controlling corrosion, wetting or adhesion properties of a surface, performing heterogeneous catalysis, extracting or purifying analytes from solutions, and for producing biosensors or biochips.

10 A conventional process for depositing a molecules on a substrate surface involves immersing the surface in a solution having an excess of molecules for forming a self-completing monolayer on the surface. A disadvantage of conventional immersion based monolayer processing techniques is that they do not allow fine
15 patterning. In addition, they are complex, expensive, slow, and inaccurate both chemically and geometrically. Conventional manufacture of DNA functionalized biochips involves spotting with DNA templates. Surface tension in the spot is relied upon to define spot geometry. Drying effects quickly change concentration
20 in the spot and render control difficult.

Another conventional process for depositing molecules on a surface involves printing the monolayer on the surface from a stamp such as a stamp made in poly dimethyl siloxane (PDMS). Printing allows patterning of the surface with minute amounts of
25 molecules. However, there is a variable transfer associated with such printing. Coverage at a given spot on the surface depends on both inking density and printing efficiency. This is particularly problematical where molecules can only be placed as a monolayer on the stamp in, for example, printing of catalysts or biological

molecules. Here, contrast between a passive and a functional
surface is provided by the presence or absence of a single
molecule. A single missing molecule produces a defect.
Conventionally, transfer of monolayers from a stamp to a surface
5 is not free of defects and transfer ratios vary from one print
cycle to the next. This is undesirable for mass production
environments. See, for example, A. Bernard et al., "Microcontact
Printing of Proteins", Adv. Mater. 2000 (12), 1067 (2000).

So-called biochip or micro array technology is increasingly
10 important in applications such as genetic analysis, including
examination of gene activity and identification of gene
mutations. Genetic information can be used to improve drug
screening, diagnostics, medication, and identification. A typical
biochip for such an application comprises a miniature array of
15 gene fragments or proteins attached to a glass surface. Typically
a hybridization reaction between sequences on the surface of such
biochips and a fluorescent sample is used for the analysis.
Following hybridization, biochips are typically read with
fluorescence detectors, permitting the fluorescent intensity of
20 spots on the surface to be quantified. Protein markers, viruses,
and protein expression profiles can be similarly detected via
protein specific capture agents. Conventional methods for
patterning biological molecules on biochips are described in M.
Schena, "Micro array Biochip Technology", Eaton Publishing,
25 Natick MA, (2000) and G. Ramsay, "DNA chips: State of the Art",
Nature Biotech, 16, 40 (1998). Conventional methods include
sequential and parallel patterning techniques. The sequential
techniques serially address spots on the surface. These
techniques include: pipetting; capillary printing; ink jet
30 printing; and, pin spotting. The parallel techniques pattern
multiple areas of molecules onto the surface simultaneously.
These techniques include: microfluidic network delivery;

capillary array printing; and, microcontact printing. Microcontact printing involves inking a patterned stamp. Such inking may be performed via a microfluidic network.

Deoxyribonucleic acid (DNA) may be applied to a biochip surface
5   for some applications. The information encoded in DNA establishes and maintains cellular and biochemical functions of an organism. In most organisms, DNA is an extended double stranded polymer. The sequence of deoxyribonucleotides of one DNA is complementary to those of the other strand. This enables new
10 DNA molecules to be synthesized with the same linear array of deoxyribonucleotides in each strand as an original DNA molecule. This process is generally referred to as DNA replication. The DNA code is made up from four bases: adenine (A), guanine (G), cytosine (C), and thymine (T). A nucleotide consists of one of
15 the four organic bases, a five carbon sugar (pentose), and a phosphate group. The phosphate group and organic base are attached to the 5′ carbon and 1′ carbon atoms of the sugar moiety, respectively. The sugar of DNA is 2′deoxyribose because it has a hydroxyl group only on the 3′ carbon. The nucleotides of
20 DNA are joined by phoshodiester bonds with the phosphate group of the 5′ carbon of one nucleotide linked to the 3′ OH of the deoxyribose of the sugar of the adjacent nucleotide. A polynucleotide thus has a 3′ OH at one end (3′end) and a 5′ phosphate group at the other end (5′end). DNA forms a double
25 stranded helix with bases A pairing with T and bases G pairing with C via two and three hydrogen bonds, respectively. The two strands of a duplex DNA run in opposite directions, by convention double stranded DNAs are always written with the 5′ end of the upper strand on the left. During the enzymatic replication
30 process, the phosphate of the added nucleotide is linked to the 3′ OH of the existing sequence. Thus, DNA is always replicated from 5′ to 3′ direction as described in <u>B.R. Glick, et al.,</u>

"Molecular Biotechnology: Principles and Applications of
Recombinant DNA", American Society for Microbiology, Washington
1998.

Manufacture of DNA functionalized biochips conventionally
5 involves sequential inking of spots with a different DNA template
to from DNA targets. This is complex, slow and thus expensive
process.
Conventionally, gene analysis was performed by hybridization of
labeled probes to the DNA targets that were passively adsorbed to
10 support surfaces such as nitrocellulose, nylon membranes, or
lysine coated glass slides. Covalent linkage of DNA to the
surface provides stable attachment under hybridization
conditions. DNA oligomers can be attached or synthesized in situ
from either the 3'-end or the 5'-end. Processes for attaching
15 5'-end oligonucleotides to glass include: an epoxy opening
reaction on epoxy silane derivatized glass such as described in
K.L. Beattie et al. Clin. Chem.41, 700-706 (1995);
5'-succinylated target oligonucleotides immobilized onto amino
derivatized glass such as described in Joos, B. et al., Anal.
20 Biochem. 247, 96-101 (1997); and, 5'-disulfide modified
oligonucleotides bound via disulfide bonds onto thiol
derivatized glass such as described in Rogers, Y.H. et al., Anal.
Biochem. 266, 23-30 (1999). Other processes use cross linkers
such as pehyldiisocyanate, maleic anhydride, or carbodiimides and
25 are described, for example, in Chrisey, L.A.et al., Nucleic Acids
Res. 24, 3031-3039 (1996), O'Donnell, M.J. et al., Anal. Chem.
69, 2438-2443 (1997), Chee, M., et al., "Accessing genetic
information with high-density DNA arrays", Science 274, 610-614
(1996). An overview of attachment chemistries is published in
30 G.T. Hermanson, "Bioconjugate Techniques", Academic Press, San
Diego, 1996. Reproducible chemisorption of oligomers in
particular are described in: Adessi,C. et al. Nucleic Acid Res.

28 (e87) 1-8 (2000); Kawashima, E., et al., "Method of nucleic acid amplification", WO 98/44151; and, Adessi, C., et al., "Methods of nucleic acid amplification and sequencing", WO 00/18957.

5   The polymerase chain reaction (PCR) is an in vitro technique permitting exponential amplification of a specific ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) region lying between two regions of known DNA sequence. Conventional applications of PCR include: gene characterization; cloning; DNA diagnostics for

10 pathogen detection; identifying mutations responsible for inherited diseases; and, DNA fingerprinting. PCR amplification is achieved via oligonucleotide primers known as ampliprimers. These are short, single stranded DNA molecules which are complementary to the ends of a defined sequence of DNA template. The primers

15 are extended in 3' directions on single stranded denatured DNA by a thermostable DNA polymerase in the presence of deoxynucleoside triphosphates (dNTPs) under suitable reaction conditions. Strand synthesis can be repeated by heat denaturation of the double stranded DNA, annealing of primers by cooling the mixture and

20 primer extension by DNA polymerase at a temperature suitable for enzyme reaction. Each repetition of strand synthesis comprises a cycle of amplification. Each new DNA strand synthesized becomes a template for any further cycle of amplification. The amplified target DNA is thus amplified exponentially. For further

25 information relating to PCR, see C.R. Newton et al., "PCR", Bios Scientific Publishers, Oxon, U.K. 2000, E. Southern et al. "Molecular Interactions on Microarrays", Nature Genetics 21, 5 (1999); U. Maskos et al., "Oligonucleotide hybridizations on glass supports" Nucleic Acid Res. 20(7), 1679-1684 (1992); Z. Guo

30 et al., "Direct Fluorescence Analysis of Genetic Polymorphisms by Hybridization with Oligonucleotide Arrays on Glass Supports" Nucleic Acid Res. 22 (24), 5456-5465 (1994); J. Lamture et al.,

"Direct Fluorescence of Nucleic Acid Hybridization on the Surface
of a Charge Coupled Device" Nucleic Acid Res. 22(11), 2121-2125
(1994); M. Sjoeroos et al., "Solid-Phase PCR with Hybridization
and Time-Resolved Fluorometry for Detection of HLA-B27", Clinical
5 Chem. 47(3), 498 (2001).

All of the above mentioned methods to pattern monolayers on
surfaces have strong limits and patterns cannot be optimized
after the patterning step. It would be desirable to solve such
problems and have printing processes that consume less ink, thus
10 requiring less frequent inking and allowing faster operation due
to shorter contact times being needed for molecular transfer.

In accordance with the present invention, there is now provided a
method for producing a monolayer of molecules on a surface, the
method comprising: loading a stamp with seed molecules;
15 transferring seed molecules from the stamp to the surface; and,
amplifying the seed molecules via an amplifying reaction to
produce the monolayer.

In a preferred embodiment of the present invention, the
transferring comprises transferring a fraction of the seed
20 molecules loaded on stamp to the surface. Preferably, this is
achieved by the transferring comprising adsorbing the seed
molecules to the stamp and adsorbing the seed molecules to the
surface, the adsorption of the seed molecules to the stamp being
stronger than the adsorption of the seed molecules to the
25 molecules to the surface. The amplifying may comprises an in
vitro translation system to produce a monolayer of protein. The
seed molecules may comprise a catalyst center for electroless
deposition. The method may include binding a catalyst to the seed
molecules for electroless deposition. In a preferred application
30 of the present invention, the monolayer protects the surface from

etchants. In a particularly preferred embodiment of the present invention, the monolayer comprises DNA. Preferred examples of the method further comprise repeating the transferring and amplifying on plural surfaces before reloading the stamp with seed
5 molecules. The present invention also extends to a biosensor comprising surface treated with a method as herein before described.

In a preferred embodiment of the present invention, there is provided a surface treatment method comprising: transferring an
10 incomplete molecular monolayer to the surface; and, using the incomplete layer as the basis for completing the monolayer by an amplifying reaction. The amplifying reaction, which may involve immersion of the surface in solution, is preferably selective of predefined zones of the surface to prevent unwanted surface
15 coverage. Reaction induced enlargement of the zones is preferably limited to avoid excessive distortion of surface patterning. In particularly preferred embodiments of the present invention, the process is self completing, thus solving the aforementioned problem of variable transfer ratio. This allows more prints
20 between re-inking, to conserve ink, and to print faster, without significantly distorting surface patterning. A subsequent reaction may convert printed species to different species which may be unprintable by other techniques. The present invention is particularly although not exclusively useful for amplifying
25 sparse monolayers such as sparse DNA monolayers.

Examples of amplification schemes for completing the monolayer include linear amplification, exponential amplification such as PCR amplification, and directional amplification. Advantageously, such schemes are generally independent from patterning methods.
30 Linear amplification is useful for completing defective monolayers. However, exponential amplification is preferred for

building monolayers from sparse molecules because it is
relatively fast in execution. Directional amplification,
intrinsically directional or dependent on application of an
external field, permits formation of nanostructures bridging
5 small gaps. Such amplification schemes can be implemented in
inorganic, organic, and biological systems. Each of these schemes
will be described in further detail shortly. Advantageously,
these schemes can be implemented using DNA oligomers. DNA may be
detected and amplified using a copying process such as PCR. It is
10 desirable to amplify multiple DNAs in parallel in the interests
of mass production. In a particularly preferred embodiment of the
present invention, parallel amplification is combined with soft
lithography printing to provide mass production simplicity and
reduced cost. Specifically, oligomers are patterned onto a
15 surface via a stamp. The patterning quality depends on the
efficiency of chemisorption of capture oligomers on the stamp and
of primer oligomers on the surface. The template oligomer
preferably has a controlled but reversible hybridization with
stronger bonding to the capture oligomer on the stamp than to the
20 primer oligomer on the surface. Alternatively, capturing of
templates on the stamp can be achieved nonspecifically using
polylysine. For efficient surface bound PCR, oligomers preferably
have spacer sequences (9T), are preferably bound on a
biocompatible spacer layer (PEG), and preferably have a
25 thermostable covalent crosslink retaining them on the surface
during thermal cycling.

In a particularly preferred embodiment of the present invention,
DNA printed from a spot feature on a stamp is reduced in amount
for each printing cycle. This allows repetitive use of the stamp
30 without re-inking. The number of printing cycles possible is
proportional to the inverse fraction transferred during each
printing cycle. Printing of less than 0.1% per printing cycle for

example allows the reuse of the stamp around 1000 times before replacement or re-inking. Printing of more than 25 DNA molecules per square micrometer is possible. This is sufficient for use in PCR amplification using surface bound primers. PCR replication

5 with a common primer region and a variable sequence allows replication of many thousands of different molecules in parallel.

In a preferred embodiment of the present invention, the seed molecules are directionally amplified to form conductive structures. Directionally amplified seed molecules may be

10 electroplated with a metal. The directional amplification may be controlled by the geometry of the seed molecule. Alternatively, the directional amplification may be controlled by application of an external force. Examples of external forces applicable include: electrical force; magnetic force; and, hydrodynamic

15 force.

Preferred embodiments of the present invention will now be described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 is a block diagram of a surface treatment process

20 embodying the present invention;

Figure 2A is a block diagram of linear amplification;

Figure 2B is a block diagram of exponential amplification;

Figure 2C is a block diagram of directional amplification;

Figures 3A to 3H are block diagrams of steps in a method for PCR

25 surface primer amplification;

Figures 4A to 4E are block diagrams of steps in a method for PCR soluble primer amplification; and,

Figures 5A to 5E are cross sectional view of a stamping operation for a surface treatment methods embodying the present invention.

5 Referring first to Figure 1, in a preferred embodiment of the present invention, there is provided a method for forming a molecular monolayer on surface 1. The method comprises transferring a seed layer of molecules 3,4 to the surface via a stamp 2. The seed layer comprises a molecular monolayer sparsely
10 populated with molecules 3,4. The seed layer deposited on the surface 1 is then grown by an amplifying reaction to complete the monolayer on the surface 1. Different types of molecules 3,4 may be disposed on different active zones 5,6 of the stamp 2. This technique solves the problem of incomplete molecular transfer
15 from the stamp 2 by transferring at least a catalytic amount of molecules and thereafter amplifying the molecules printed on the surface 1 to saturation density. In detail, at step 50, the stamp 2, partially inked with molecules 3,4, is brought into contact with the surface 1. In this example, a first group of molecules 3
20 is inked onto feature 5 of the stamp 2 and a second group of molecules 4 is inked onto feature 6 of the stamp 6. Molecules 3 and 4 may be of different species. The stamp 2 transfers a fraction of the inked molecules 3,4 to the surface 1. At step 55, an amplifying reaction is performed. The amplifying reaction
25 amplifies the printed molecules 3,4 to produce complete monolayers. Meanwhile, at step 51, the stamp 2 is reused to print another fraction of the remaining molecules 3,4 onto another surface 1'. Again, at step 55, the amplifying reaction amplifies the molecules 3,4 printed on the other surface 1' to produce
30 complete monolayers. Similarly, at step 52, the stamp 2 is reused again to print yet another fraction of the remaining molecules

3,4 onto yet another surface 1''. Again at step 55, the
amplifying reaction amplifies the molecules printed on the
surface 1'' to produce complete monolayers. In a batch process
additional surfaces can be similarly treated until, on the Nth
5 print, there are no molecules 3,4 remaining on the stamp 2. At
step 53, the stamp can then be reinked and the process repeated.
The amplifying reaction at step 55 may comprise an in vitro
translation system to produce a monolayer of protein. The seed
molecules 3,4 may comprise a catalyst center for electroless
10 deposition. A catalyst may be bound to the molecules 3,4 for
electroless deposition. In a preferred application of the present
invention, the monolayers protect the surface 1 from etchants.
The molecules 3,4 may be DNA oligomers.

Amplifying of printable DNA molecules may also be done on the
15 stamp 2 followed by transfer of the complete monolayer. This
method is however disadvantageous because a lithographic stamp is
a costly and thermally sensitive article. Economically, this
method is not useful because it occupies the stamp for at least
one cycle of amplification. When the amplification in done on
20 substrate surfaces, it can be done in a batch process, involving
multiple substrates printed by the same stamp.

The amplifying reaction may be a linear amplification reaction,
an exponential amplification reaction, or a directional
amplification reaction. Other amplifying reaction may be
25 possible. An example of linear amplification of an incomplete
printed monolayer of molecules having headgroup X, a backbone,
and tailgroup Y will now be described with reference to Figure
2A. Group Y provides an anchor for the V part of a molecule that
also has a group X binding to the surface 1. The reaction stops
30 at the edge of the print, where X and V have insufficient
affinity for the surface 1. In detail, at step 10, the incomplete

monolayer of the X-Y molecules is printed on the surface 1. At
step 20, the tail group Y binds to the surface 1 from solution a
molecule having a V function, a backbone and a precursor
chemisorbing group (X). At step 30, the chemisorbing group (X) is
5 deprotected to expose X. At step 40, this leads to chemisorption
of the bound molecule to the surface. Chemisorption occurs only
if the surface 1 is not already covered. The binding,
deprotecting and amplifying steps can be repeated to
progressively amplify the initial print until the surface 1 is
10 covered. This process is thus self-limiting. It stops when the
monolayer is complete or when no empty sites on the surface 1
remain in reach of molecules bound to Y tails. If the monolayer
is patterned, the process produces negligible spreading, thus
maintaining resolution and the maximum of molecules added after m
15 amplification cycles is m, corresponding to linear amplification.

Referring now to Figure 2B, in an example of exponential
amplification of an incomplete printed monolayer comprising
molecules having headgroup X and tailgroup Y, a complex or
20 mediator M allows binding of more molecules and promotes
adsorption to the surface 1. Since the newly bound tail groups Y
also act as binding sites for M, the number of molecules N
increases at 2^N. This process is similar to linear amplification.
However, after the printing step 10, Y tails are complexed at
25 step 21 with an atom or molecule M. At step 22, a second molecule
having a headgroup (X) and tail group Y is bound to M. At step
31, the headgroup (X) of the second molecule is deprotected to X
and chemisorbed to the surface 1. Amplification thereafter
proceeds by repeating the first binding step 21, second binding
30 step 22, and deprotection step 31. Every molecule deposited on
the surface can serve to amplify the monolayer further.

Directional amplification will now be described with reference to
Figure 2C. Direction amplification is similar or faster than
linear amplification except that now, at step 32, the second
binding molecule is chemisorbed onto the surface in a directional
5 manner which is set by the geometry of the complex YMY. Seed
molecules may be directionally amplified to form conductive
structures. Directionally amplified seed molecules may be
electroplated with a metal. The directional amplification may be
controlled by the geometry of the seed molecule. Alternatively,
10 the directional amplification may be controlled by application of
an external force. Examples of external forces applicable
include: electrical force; magnetic force; and, hydrodynamic
force.

The three amplification schemes can be used to coat surfaces with
15 DNA oligomers. Exponential amplification in particular is
especially useful for derivating surfaces with DNA. PCR
amplification is an example of an exponential amplification
scheme. In a preferred embodiment of the present invention, DNA
molecules are sparsely printed from a stamp onto a surface as
20 herein before described with reference to Figure 1 and then
amplified to produce a complete monolayers via solid phase PCR.
The three amplification schemes herein before described are also
suited to amplify monolayers in general and in particular printed
monolayers or may be used to grow molecular structures along
25 preferential directions of a surface to make nanostructures, for
example.

A method for surface primer PCR replication will now be described
with reference to Figures 3A to 3H. Referring to Figure 3A,
template oligomers 8 are initially held on the stamp 2 by capture
30 oligomers 7. The stamp 2 is then brought into contact with the
surface on which first and second primer oligomers 9 are

immobilized. A fraction of the templates 8 hybridize with the primers 9 and are thus transferred from the stamp 2 to the surface 1. Turning to Figure 3B, a PCR mix 11 comprising DNA polymerase and the four PCR nucleotides (dNTPs) in a buffer

5   solution of phosphate buffered saline (PBS) is then added. Each immobilized primer hybridized to a DNA template is amplified by DNA polymerase in the PCR mix 11 to full length on the surface 1. This produces a synthesized complementary or duplex DNA strand. Referring to Figure 3C, heat is now applied. The duplex DNA

10   strand melts and rehybridizes with another primer 9 on the surface 1 to produce a bridged molecule. Referring now to Figure 3D, further application of heat melts the bridged molecule. Two duplex DNA strands bound to the surface 1 are thus produced. Referring now to Figure 3E, temperature is reduced. Each of the

15   two duplex strands rehybridizes with a matching primer 9 and the primers 9 are extended. Two bridged molecules are thus produced. Referring now to Figure 3F, further application of heat melts the bridged molecules. Now four duplex DNA strands are bound to the surface 1. Referring now to Figure 3G, the melting and

20   rehybridizing steps are repeated until all primers 9 are elongated. Turning to Figure 3H, the complementary molecules are then cleaved from the surface 1 chemically or via a restriction enzyme.

In a modification of the PCR technique herein before described,
25   linear amplification of DNA can be achieved by applying antisense DNA primers in solution. This modification will now be described with reference to Figures 4A to 4E. Referring to Figure 4A, template oligomers 8 are initially held on the stamp 2 by capture oligomers 7. The stamp 2 is then brought into contact with the

30   surface with the first primers 9 only are immobilized. A fraction of the templates 8 hybridize with the immobilized primers 9 and are thus transferred from the stamp 2 to the surface 1. Turning

to Figure 4B, a PCR mix 11 comprising DNA polymerase and the four
PCR nucleotides (dNTPs) in a buffer solution of phosphate
buffered saline (PBS) is then added. Each immobilized primer
hybridized to a DNA template is amplified by the PCR mix 11 to
5   full length on the surface 1. This produces a synthesized
complementary or duplex DNA strand. Referring now to Figure 4C,
the second primer 12 is added in solution and heat applied. The
synthesized strand is melted and rehybridized. Referring to
Figure 4D, a second generation complementary strand is then
10 synthesized. Referring to Figure 4E, the synthesis is repeated,
until all oligomers are extended. This method is preferable
performed in a sealed container to avoid cross contamination of
the generated and detached template strands.

In preferred embodiments of the present invention, a fraction or
15 catalytic amount of molecules is transferred from a stamp 2 to
the surface 1 via mechanical contact with the surface 1 in a dry
or wet state. This is achieved by the molecules to be transferred
having a stronger bond to the stamp 2 than to the surface 1.

It is desirable to employ a controllable chemisorption protocol
20 that deters nonspecific adsorption on the surface 1 or on the
stamp 2. An example of such a reaction involves preparation of a
heterobifunctional reagent such as NHS-PEG-triethoxysilane from
APTS and a homobifunctional PEG such as (a,w)NHS-PEG 2000, Rapp
Polymere which is exposed to a glass surface by filling a gap
25 between glass slides at elevated temperature. Chemisorption may
be performed by filling PDMS microfluidic systems applied to the
NHS-activated surface with aqueous solutions of
amino-functionalized oligomers.

PCR amplification can be performed using two surface bound
30 primers as herein before described with reference to Figure 3, or

using one surface bound primer and one soluble primer as herein before described with reference to Figure 4. However, in the latter case, traces of intermediate templates can diffuse away from their synthesis location and diffuse into adjacent areas. It

5 is therefore desirable to confine soluble primer based PCR amplification. For biosensor applications, only one sense strand and not the antisense strand should be present on the surface 1. The other strand is therefore preferably either cleaved from the surface or stopped from chemically attaching by use of, for

10 example, soluble primers.

Referring back to Figure 1, as indicated earlier, active zones 5,6 of the stamp 2 are selectively coated in a preferred embodiment of the present invention with a capture molecule and an inking molecule such as a template strand. Such selective

15 coating may be performed via a range of different methods, including: pipetting; capillary printing; ink jet; and, pin spotting, as herein before described. Other coating techniques, such as application of ink via a microfluidic network or via a stencil with selective openings are equally possible. A

20 topographically patterned stamp usually produces a more accurate pattern than a flat stamp patterned with ink. This is because the active zones 5,6 are topographically separated. The delimitation is effective both when active zones 5,6 are protruding and when they are recessed.

25 Referring now to Figure 5, in a particularly preferred embodiment of the present invention, the stamp 2 comprises a body 41 having plurality of active zones in the form of pores 42. The pores 42 may be open to each end or closed at one end. Referring to Figure 5B, each pore 42 is filled with a hydrophilic polymer gel matrix

30 43. Referring to Figure 5C, each pore 42 may be filled with molecules 44 of different species. A stencil 45 or microfluidic

network may be employed to mask each pore 42 from the other pores during filling, thus preventing cross contamination of the pores 42. The filling may be by diffusion or by an electric field. Referring to Figure 5D, in operation, the stamp 2 contacts the

5 surface 1. Molecules are printed on the surface 1 from the gel in the pores 42. Referring to figure 5E, printed areas 46 of molecules are left on the surface 1.

In a particularly preferred embodiment of the present invention, the pores 42 are each loaded with a different species of template

10 DNA. By uptake of water 47 or buffer, the gel 43 swells to its equilibrium in a 100% humidity environment. The gel 43 thus protrudes beyond the stamp surface. The stamp 2 may be stored in a humid environment to prevent the subsequent drying of the gel 42. With a loading of around 1W% DNA in the gel, millions of

15 surfaces may be printed from the same stamp 2 with a catalytic amount of DNA transferred each time. Subsequent amplification of the catalytic seed layers can be employed to complete the DNA monolayers. Refilling of the stamp 2 need be performed only when the stamp 2 is no longer able to transfer a seed layer. To

20 deposit the seed layers, the stamp 2 is brought into contact with the surface 1 to transfer the desired amount of seed molecules. The stamp 2 need not be immersed in liquid, thereby reducing printing complexity. The gel 43 permits full hydration of molecules thus enhancing chemisorption of the molecules to the

25 surface 1. The gel 43 is permeable, thus allowing trapped water to escape. This avoids separation of the printing surfaces in the presence of a third medium. DNA may be held on the surface of a pore 42 rather than within the gel 43. Here, it is desirable to effect contact between the stamp 2 and the surface 1 in a third

30 medium with the pores 42 isolated from each other. After contact, the temperature can be increased to promote the dissociation of

traces of template DNA strands from the stamp 2 and deposition on the surface 1.

In a preferred embodiment of the present invention, the active zones 5,6 of the stamp 2 herein before described with reference
5 to Figure 1 are each provided with oligomer for capturing template DNA strands. The DNA strands are then exposed to the surface 1 such that only a small fraction, typically <0.1% of a monolayer of DNA strands are transferred. This is achieved by providing hybridizing anchors with shorter length on the surface
10 1. The number of DNA strands transferred may be around 25 per square micrometer with a transfer efficiency of 0.1% and a DNA diameter of 2 nm. The stamp 2 can thus be used for several hundred printing operations before reinking is needed. The density of DNA strands on the surface is then brought to
15 saturation via the herein before described PCR amplification scheme involving surface bound primers. The active zones 5,6 on the stamp 2 may range from microns to millimeters in size.

Patterning of templates for PCR onto the primer may also be achieved by microfluidic networks. Use of solutions with low
20 concentration of templates and conditions unfavorable to fast binding of templates to the surface permit conservation of templates and patterning of homogeneous areas on the surface. Replication can also be applied to repair of defects in printed monolayer and to situations where an autocatalytic center is
25 printed and a catalytic reaction is started.